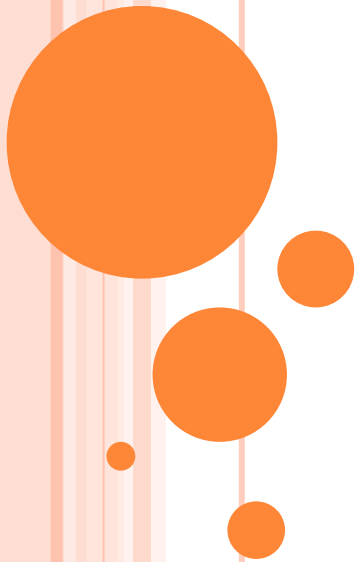


# DNA REPLICATION

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# JAMES WATSON AND FRANCIS CRICK

- Watson and Crick proposed the hypothesis of semi conservative replication soon after publication of their 1953 paper on the structure of DNA



# FUNDAMENTAL RULES

- Occurs during interphase of cycle
- Semi conservative- one new strand and one old strand
- Begins at an Origin
- Proceeds Bidirectionally
- Proceeds in a 5' → 3' direction
- Is Semi discontinuous- one strand is synthesized continuously and the other discontinuously



# REQUIREMENTS

- Template
- Pre priming complex
- Primase : Synthesizes RNA primers
- Ribonucleotides
- DNA polymerases
- Deoxyriboneucleotides
- DNA ligase



# PRE PRIMING COMPLEX

- DNA a : binds to origin site and recognizes and denatures the DNA in the AT rich region
- DNA b( Helicase ): DNA unwinding
- DNA c :loads the DNA b protein onto the unwound region
- SSB - single-stranded DNA–binding protein :  
Binding to single-stranded DNA
- DNA topoisomerases : Relieves torsional strain generated by DNA unwinding



# DNA POLYMERASES

- enzymes that can synthesize DNA
- The fundamental reaction is a phosphoryl group transfer.
- The nucleophile is the 3-hydroxyl group of the nucleotide at the 3' end of the growing strand.
- Nucleophilic attack occurs at the *phosphorus of the incoming deoxynucleoside 5-triphosphate*
- Inorganic pyrophosphate is released in the reaction.
- $$(dNMP)_n + dNTP \longrightarrow (dNMP)_{n+1} + PPI$$

DNA

DNA Lengthened
- **Processivity** : The average number of nucleotides added before a polymerase dissociates

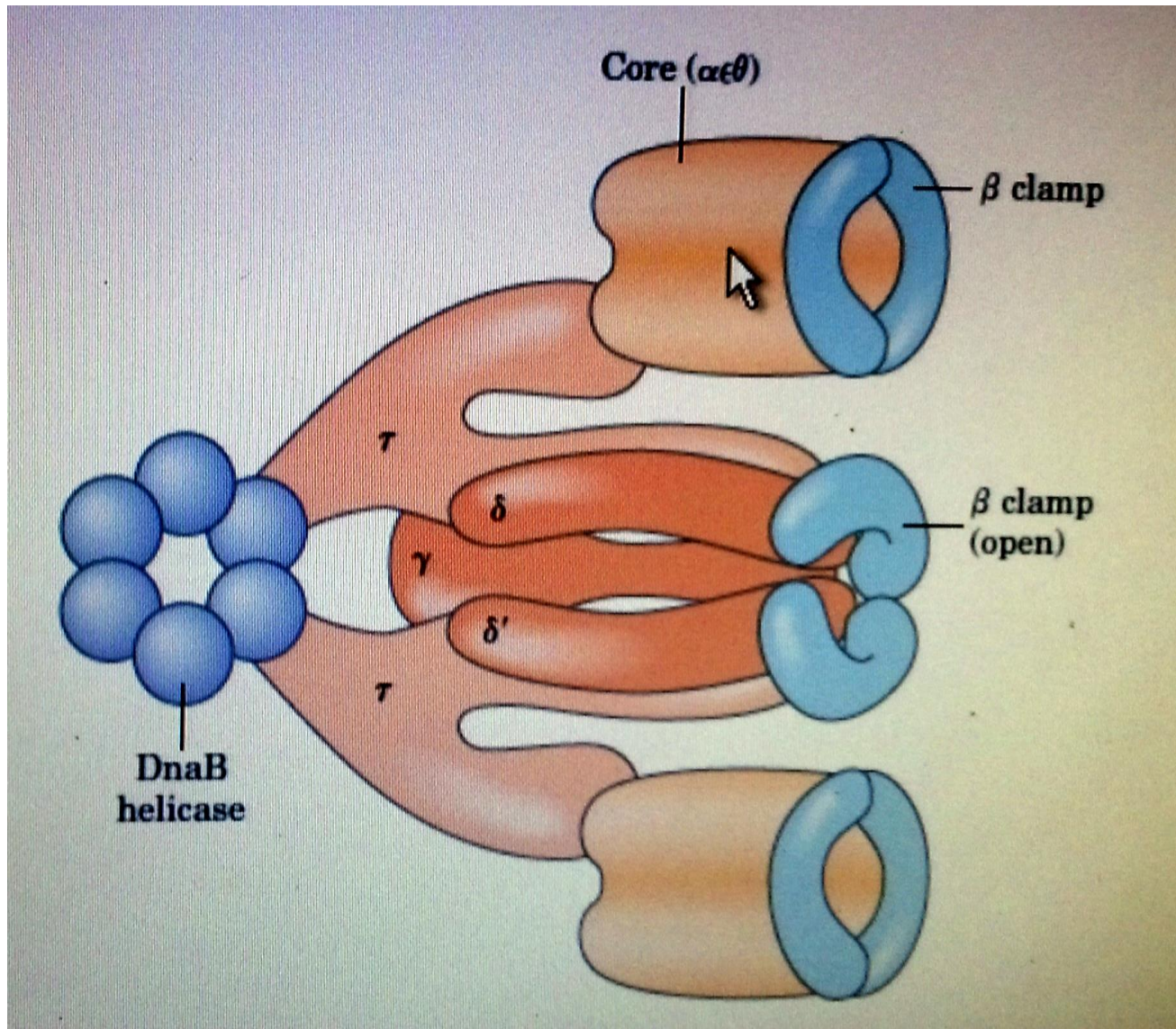


## TYPES

- *DNA polymerase I : pol A – 5' → 3' Exonuclease*  
- removal of primers
- *DNA polymerase II : pol B – DNA repair*
- *DNA polymerase III pol C –*  
-principal replication enzyme in *E. coli*.



# DNA POLYMERASES III



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- The synthesis of a DNA molecule can be divided into three stages:

- Initiation,
- Elongation,
- Termination



# INITIATION

- The *E. coli* replication origin – *ori C* :
  - is highly enriched in GATC sequences
  - conserved Consensus sequence
  - two series of short repeats: three repeats of a 13 bp sequence and four repeats of a 9 bp sequence.
- The timing of replication initiation is affected by DNA methylation and interactions with the bacterial plasma membrane.
- The *oriC* DNA is methylated by the Dam methylase, which methylates the N6 position of adenine within the palindromic sequence (5')GATC.

- 
- Dna A protein binds to the four 9 bp repeats in the origin, then recognizes and denatures the DNA in the region of the three 13 bp repeats, which are rich in AT pairs
  - requires ATP
- The DnaC protein then loads the DNA B protein onto the unwound region



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- DNA B act as helicases, unwinding the DNA bidirectionally and creating two potential replication forks.
- SSB bind cooperatively to single stranded DNA, stabilizing the separated strands and also protects DNA from nucleases
- DNA topoisomerases relieve the topological stress produced by the DnaB helicase
  - DNA topoisomerases I
  - DNA topoisomerases II



# REPLICATION FORK

Two strands –

✓ Leading strand –

- strand copied in the direction of the advancing replication fork
- synthesized continuously

✓ Lagging strand –

- strand copied in the direction away from the advancing replication fork
- synthesized discontinuously
- Okazaki fragments



# SYNTHESIS OF RNA PRIMER

- Short double stranded region consisting RNA bases paired to DNA template with a free hydroxyl group on 3' end of the RNA strand which serves as the first acceptor of deoxynucleotide by action of DNA polymerase
- Primases : specific RNA polymerase synthesizing short stretches of RNA complementary and antiparallel to DNA template
- Leading strand – only one RNA sequence
- Lagging strand – multiple RNA sequences



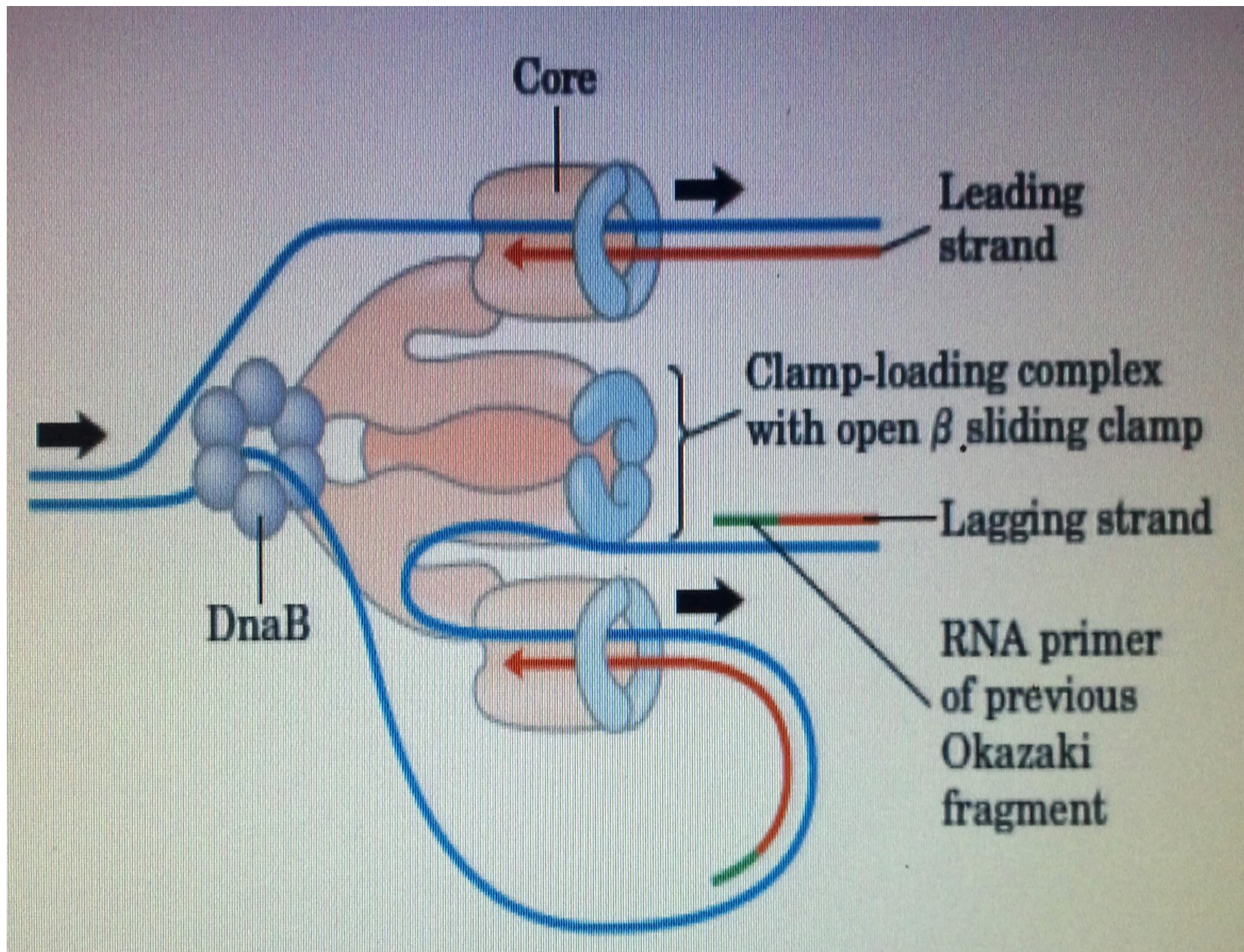
# ELONGATION

- Deoxyriboneucleotides are added to the primer by DNA polymerase III.
- Leading strand synthesis then proceeds continuously, keeping pace with the unwinding of DNA at the replication fork.
- Lagging strand synthesis is accomplished in short Okazaki fragments.



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- ***coordination of leading and lagging strand synthesis:***
  - both strands are produced by a *single asymmetric DNA polymerase III dimer, which is accomplished by **looping** the DNA of the lagging strand ,bringing together the two points of polymerization.*





# PROOFREADING

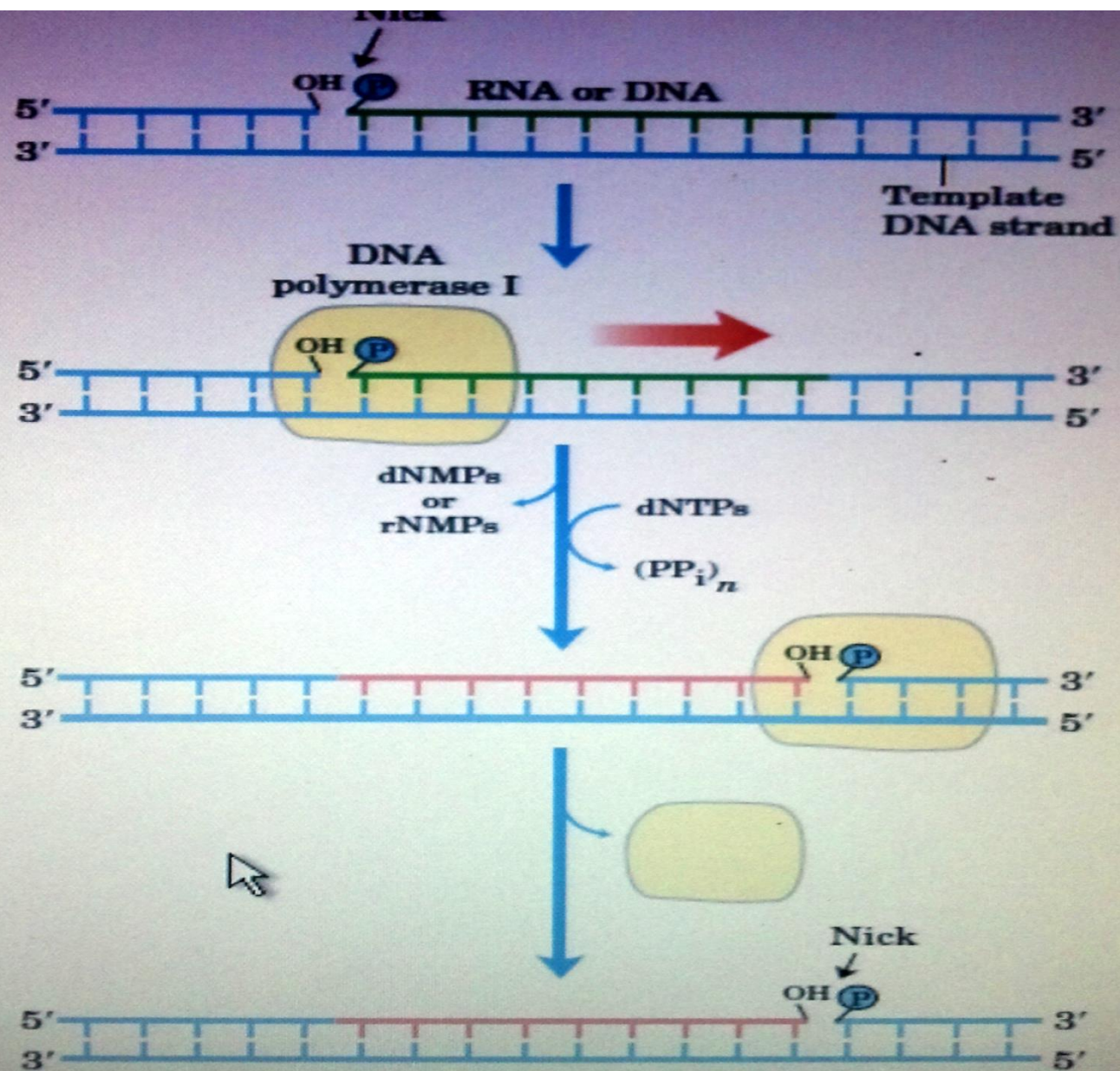
- To ensure replication fidelity
- DNA polymerase III in addition to 5' to 3' polymerase activity, it has 3' to 5' exonuclease activity (proofreading activity)



# EXCISION OF RNA PRIMERS

- RNA is excised and the gap is filled by DNA polymerase I
  - DNA polymerase I - 5' to 3' polymerase activity
    - 3' to 5' exonuclease activity
    - **5' to 3' exonuclease activity**
- Nick translation





**FIGURE 25-9** Nick translation. In this process, an RNA or DNA strand

# DNA LIGASE

- Final phosphodiester linkage between 5' phosphate group of DNA synthesized by pol 3 and 3' OH group of DNA synthesized by pol 1 is catalysed by DNA ligase
- ATP dependant



# TERMINATION

- The two replication forks of the circular *E. coli* chromosome meet at a terminus region containing multiple copies of a 20 bp sequence called Ter (for *terminus*).
- The Ter sequences function as binding sites for a protein called Tus (*terminus utilization substance*).
- The Tus-Ter complex can arrest a replication fork from only one direction.
- Only one Tus-Ter complex functions per replication cycle



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- The final few hundred base pairs of DNA between these large protein complexes are then replicated completing two topologically interlinked (catenated) circular chromosomes.
- DNA circles linked in this way are known as **catenanes**.
- Separation of the catenated circles in *E. coli* requires topoisomerase IV (a type II topoisomerase).
- The separated chromosomes then segregate into daughter cells at cell division.



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# Thank you

